PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 501872 KXR	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. **PCT/NZ2004/000222**	International filing date *(day/month/year)* 20 September 2004	Priority date *(day/month/year)* 18 September 2003
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] G01N 21/05, 33/577		
Applicant THE HORTICULTURE AND FOOD RESEARCH INSTITUTE OF NEW ZEALANDLIMITED		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of **2** sheets, as follows:
      - [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

[X]	Box No. I	Basis of the report
[ ]	Box No. II	Priority
[ ]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[ ]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[X]	Box No. VI	Certain documents cited
[ ]	Box No. VII	Certain defects in the international application
[X]	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
18 July 2005	16 August 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **ROSS OSBORNE** Telephone No. (02) 6283 2404

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NZ2004/000222

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1 (b))

   ☐ publication of the international application (under Rule 12.4)

   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

   ☐ the international application as originally filed/furnished

   ☒ the description:

   pages **1-47, 52** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☒ the claims:

   pages **48, 50,** as originally filed/furnished
   pages* as amended (together with any statement) under Article 19
   pages* **49, 51** received by this Authority on **18 July 2005** with the letter of 18 July 2005
   pages* received by this Authority on with the letter of

   ☒ the drawings:

   pages **1/7-7/7** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	**1-18**	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	**1-18**	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	**1-18**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

D1 US6342349

D2 Wu Y. et al,

NOVELTY(N), INVENTIVE STEP (IS) Claims 1-18

D1 discloses an assay device that can be used with competitive binding assays that features 'dual linkers' with examples of a binding partner bound to a solid metal surface by means of a linker and in the liquid phase a binding partner bound through a linker to a optical signaller particle for the detection of analyte. D2 discloses the use of nanoparticles and linkers of optimized length to attach to binding partners for improved sensitivity in a flow through surface plasmon resonance-based immunoassay.

None of the prior art documents disclose an flow through immunoassay for the detection of haptens featuring an analyte/antibody bound through a linker to a high mass signalling molecule and a antibody/analyte bound to a sensor through a second linker. D2 does disclose the use of a signalling particle but the signal is an optical one. The claims are therefore novel.

The applicant's arguments that it would not be obvious to combine the teaching of D1 which is a non-flow through assay which may utilise multiple linkers with the teaching of D2 which is a flow through system that utilises only one linker are accepted. All claims therefore have an inventive step.

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Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO 2004/042403	21 May 2004	3 November 2003	4 November 2002

WO 2004/042403 discloses a plasmon resonance immunoassay which uses immunogold and a solid phase with attached substrate binding antibodies but does not disclose the use of dual linkers.

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The claims are not concise, there are 6 independent claims among the 18 claims that define the invention. There does not appear to be six different substantively described variations of the invention in the description and these claims do not appear to differ in any fundamental manner. This lack of conciseness also leads to a lack of clarity about the precise ambit of the invention and matter should be addressed by reducing the number of independent claims.

The description only supports flow though assays where the signalling is done on the basis of mass, such as plasmon resonance assays and all the claims should be clearly limited to this type of assay. Otherwise some of the claims arguably cover dual linker assays such those described in D1 where there is a 'high mass' particle that signals on the basis of its optical properties and the options envisaged for the assays appear to include options where the sample flows through or over the detection sites.

d) detecting the amount of binding partner bound to said immobilised hapten or an analogue thereof.

3. A method for detecting a hapten in a sample comprising the steps of:

a) providing a sample potentially containing a hapten of interest;

b) providing a pre-determined amount of the hapten of interest or an analogue thereof, said hapten or analogue thereof being bound to a signaller and separated therefrom by a first linker wherein said signaller is a large protein or a nanoparticle providing a high mass signal;

c) providing a flow of the resultant mixture of a) and b) to an immobilised binding partner that specifically binds to the hapten of interest, said binding partner being bound to the surface of a sensor and separated therefrom by a second linker; and

d) detecting the amount of hapten or analogue thereof bound to said immobilised binding partner.

4. A method for detecting a in a sample using a rapid flow-through inhibition assay format comprising the steps of:

a) Providing a functionalised hapten derivative with a linking group (first linker) between the hapten molecule and its functional group;

b) Providing an immobilised hapten derivative on the surface of an optical biosensor chip;

c) Mixing high molecular weight detecting molecules with sample analytes to form immuno-complexes, and then flow-through of the mixing solution containing excess free antibodies to bind to the sensor surface;

d) Further binding enhancement performed by flowing-through onto the sensor surface with a solution containing a specially designed bio-conjugate, in which by employing a suitable linker (second linker), a moiety to specifically recognise a detecting molecule such as an antibody is linked at one end of the conjugate, and the other end of the conjugate is attached to a large protein or/and a *nano*-particle for high mass signal enhancement;

e) Rapid on-line flow-through regeneration to completely remove detecting molecules such as antibodies for multiple measurements;

f) A standard curve prepared from solutions with a series of known analyte concentrations, and the concentrations of analyte in unknown samples are then derived from the standard curve.

carbon-chain with substituted groups; (d) an amino acid chain, amino acid fragments incorporated into the chain, or multiple amino-acid fragments chain by homologation; (e) an oligoethylene glycol or a polyethylene glycol chain; (f) a chain having one or more sites of unsaturation such as alkenyl; and (g) a nucleic acid chain; or (h) a polysaccharide chain.

15. A method as claimed in any one of claims 1-14 wherein the hapten is a steroid and the linker between steroid and the surface is an oligoethylene glycol or a polyethylene glycol chain.

16. A method as claimed in any one of claims 1-15 wherein the signaller is a nanoparticle.

17. A method as claimed in any one of claims 1-16 wherein the signaller is an immunogold particle.

18. A Surface Plasmon Resonance based immunoassay format method comprising the steps:

(a) chemically immobilising a hapten or hapten conjugate onto the optical biosensor surface through a linker molecule (the second linker) with or without using a hapten attachment intermediate,

(b) mixing a fixed concentration of a binding partner - (the first linker)-nanoparticle conjugate in buffer with each of a series of standard free solution or a sample hapten solution and incubating for a few minutes,

(c) injecting the above mixture or the remaining binding partner in equilibrium solution onto the hapten - biosensor surfaces, and measuring binding partner responses,

(d) injecting regeneration buffer onto the biosensor surface to remove binding partner-(the first linker)-nanoparticle conjugate,

(e)plotting concentrations of free hapten versus average response (resonance units) of binding partner -(the first linker)-nanoparticle conjugate to provide an assay standard curve from which determining the concentration of unknown sample hapten when using the same method.